



Pending claims (03/27/03)
09/424,487
019496-006220US

RECEIVED
APR 04 2003
TECH CENTER 1600/2900

IN THE CLAIMS:

1. (previously amended) A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:

- i) selecting a quadruplet within the target nucleotide sequence;
- ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by choosing the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:
 - a) if base 4 in the quadruplet is A, then position +6 in the α -helix is Glu, Asn or Val;
 - b) if base 4 in the quadruplet is C, then position +6 in the α -helix is Ser, Thr, Val, Ala, Glu or Asn
 - c) if base 4 in the quadruplet is G, then position +6 in the α -helix is Arg or Lys;
 - d) if base 4 in the quadruplet is T, then position +6 in the α -helix is Ser, Thr, Val or Lys;
- iii) synthesizing a polynucleotide encoding the binding protein of (ii);
- iv) introducing the polynucleotide of (iii) into a cell; and
- v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

2. (previously amended) A method according to claim 1, wherein base 4 is G or T.

3. (previously amended) A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:

- i) selecting a quadruplet within the target nucleotide sequence;
- ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by choosing the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:
 - a) if base 4 in the quadruplet is G, then position +6 in the α -helix is Arg or Lys;
 - b) if base 4 in the quadruplet is A, then position +6 in the α -helix is Glu, Asn or Val;
 - c) if base 4 in the quadruplet is T, then position +6 in the α -helix is Ser, Thr, Val or Lys;
 - d) if base 4 in the quadruplet is C, then position +6 in the α -helix is Ser, Thr, Val, Ala, Glu or Asn;
 - e) if base 3 in the quadruplet is G, then position +3 in the α -helix is His;
 - f) if base 3 in the quadruplet is A, then position +3 in the α -helix is Asn;
 - g) if base 3 in the quadruplet is T, then position +3 in the α -helix is Ala, Ser or Val;
 - h) if base 3 in the quadruplet is C, then position +3 in the α -helix is Ser, Asp, Glu, Leu, Thr or Val;
 - i) if base 2 in the quadruplet is G, then position -1 in the α -helix is Arg;
 - j) if base 2 in the quadruplet is A, then position -1 in the α -helix is Gln;
 - k) if base 2 in the quadruplet is T, then position -1 in the α -helix is His or Thr;

l) if base 2 in the quadruplet is C, then position -1 in the α -helix is Asp or His;

m) if base 1 in the quadruplet is G, then position +2 is Glu;

n) if base 1 in the quadruplet is A, then position +2 Arg or Gln;

o) if base 1 in the quadruplet is C, then position +2 is Asn, Gln, Arg, His or Lys;

(p) if base 1 in the quadruplet is T, then position +2 is Ser or Thr

iii) synthesizing a polynucleotide encoding the binding protein of (ii);

iv) introducing the polynucleotide of (iii) into a cell; and

v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

4. (previously amended) A method according to claim 3, wherein the each zinc finger has the general primary structure

X^a Cys X_{2-4} Cys- X_{2-3} -Phe- X^c -X-X-X-X-Leu-X-X-His-X-X- X^b His-linker (SEQ ID NO: 3)

-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a , X^b and X^c) is any amino acid.

5. (previously amended) A method according to claim 4 wherein X_a is Phe/Tyr-X or Pro-Phe/Tyr-X.

6. (previously amended) A method according to claim 5 wherein X_{2-4} is selected from any one of:
Ser-X, Glu-X, Lys-X, Thr-X, Pro-X and Arg-X.

7. (previously amended) A method according to claim 4 wherein X^b is Thr or Ile.

8. (previously amended) A method according to claim 4 wherein X^{2-4} is Gly-Lys-Ala, Gly-Lys-Cys, Gly-Lys-Ser, Gly-Lys-Gly, Met-Arg-Asn or Met-Arg.

9. (previously amended) A method according to claim 4 wherein the linker is Thr-Gly-Glu-Lys (SEQ ID NO: 4) or Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 5).

10. (previously amended) A method according to claim 4 wherein position +9 is Arg or Lys.

11. (previously amended) A method according to claim 4 wherein positions +1, +5 and +8 are not occupied by any one of the hydrophobic amino acids, Phe, Trp or Tyr.

12. (previously amended) A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues Lys, Thr and Gln respectively.

13. (previously amended) A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class which binds a target nucleic acid sequence, comprising the steps of:

a) selecting a model zinc finger domain from the group consisting of naturally occurring zinc fingers and consensus zinc fingers; and

b) mutating the finger according to the rules set in any one of claims 1 to 3.

14. (previously amended) A method according to claim 13, wherein the model zinc finger is a consensus zinc finger whose structure is selected from the group consisting of the consensus structure Pro-Tyr-Lys-Cys-Pro-Glu-Cys-Gly-Lys-Ser-Phe-Ser-Gln-Lys-Ser-Asp-Leu-Val-Lys-His-Gln-Arg-Thr-His-Thr-Gly (SEQ ID NO: 6), and the consensus structure Pro-Tyr-Lys-Cys-Ser-Glu-Cys-Gly-Lys-Ala-Phe-Ser-Gln-Lys-Ser-Asn-Leu-Thr-Arg-His-Gln-Arg-Ile-His-Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 7).

15. (previously amended) A method according to claim 13 wherein the model zinc finger is a naturally-occurring zinc finger whose structure is selected from one finger of a protein selected from the group consisting of Zif 268, GLI , Tramtrack and YY1.

16. (original) A method according to claim 15 wherein the model zinc finger is finger 2 of Zif 268.

17. (previously amended) A method according to claim 3 wherein the binding protein comprises two or more zinc finger binding motifs, placed N-terminus to C-terminus.

18. (previously amended) A method according to claim 14, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence Met-Ala-Glu-Glu-Lys-Pro (SEQ ID NO: 8).

19. (previously amended) A method according to claim 13 wherein the nucleic acid binding protein is obtained by recombinant nucleic acid technology, the method comprising the steps of:

- a) preparing a nucleic acid coding sequence encoding two or more model zinc finger domains, placed N-terminus to C-terminus;
- b) inserting the nucleic acid sequence into a suitable expression vector; and
- c) expressing the nucleic acid sequence in a host organism in order to obtain the nucleic acid binding protein.

20. (previously amended) A method according to claim 3 comprising the additional steps of subjecting the nucleic acid binding protein to one or more rounds of randomisation and selection in order to improve the characteristics thereof.

21. (original) A method according to claim 20, wherein the randomisation and selection is carried out by phage display technology.

22. (previously amended) A method according to claim 21, comprising the steps of:

a) preparing a nucleic acid construct which express a fusion protein comprising the nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

b) preparing further nucleic acid constructs which express a fusion protein comprising a selectively mutated nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

c) causing the fusion proteins defined in steps (a) and (b) to be expressed on the surface of bacteriophage transformed with the nucleic acid constructs;

d) assaying the ability of the bacteriophage to bind the target nucleic acid sequence and selecting the bacteriophage demonstrating superior binding characteristics.

23. (previously amended) A method according to claim 20 wherein the nucleic acid binding protein is selectively randomised at any one of positions +1, +5, +8, -1, +2, +3 or +6.

24. (original) A method according to claim 23, wherein, in the nucleic acid binding protein, position +6 of a zinc finger and positions -1, +1, +2 and +3 of an adjacent zinc finger are randomised.

25. (previously amended) A method for determining the presence of a target nucleic acid molecule, comprising the steps of:

- a) preparing a nucleic acid binding protein by the method of claim 3 which is specific for the target nucleic acid molecule;
- b) exposing a test system comprising the target nucleic acid molecule to the nucleic acid binding protein under conditions which promote binding, and removing any nucleic acid binding protein which remains unbound;
- c) detecting the presence of the nucleic acid binding protein in the test system.

26. (original) A method according to claim 25, wherein the presence of the nucleic acid binding protein in the test system is detected by means of an antibody.

27. (previously amended) A method according to claim 25 wherein the nucleic acid binding protein, in use, is displayed on the surface of a filamentous bacteriophage and the presence of the nucleic acid binding protein is detected by detecting the bacteriophage or a component thereof.

28. (previously amended) A synthetic nucleic acid binding protein whose design incorporates a method according to claim 3.

29. (original) A nucleic acid encoding a nucleic acid binding protein according to claim 28.

30. (original) A host cell transformed with a nucleic acid according to claim 29.

31. (canceled)

32. (previously added) The method of claim 3, wherein a plurality of overlapping quadruplets are selected within the target sequence.